

We claim:

1. A vector comprising the following operably linked DNA fragments:
  - an origin of replication allowing replication in a recipient cell (1), preferably in bacteria; particularly in *Escherichia coli*;
  - a selectable marker region (2) capable of being expressed in said recipient cell; and
  - a chimeric DNA construct comprising in sequence:
    - a promoter or promoter region (3) capable of being recognized by RNA polymerases of a eukaryotic cell;
    - a first recombination site (4), a second recombination site (5), a third recombination site (6) and a fourth recombination site (7);
    - a 3' transcription terminating and polyadenylation region (8) functional in said eukaryotic cell;

wherein

said first recombination site (4) and said fourth recombination site (7) are capable of reacting with a same recombination site, preferably are identical; and  
 said second recombination site (5) and said third recombination site (6), are capable of reacting with a same recombination site, preferably are identical;

and wherein

said first recombination site (4) and said second recombination site (5) do not recombine with each other or with a same recombination site; or  
 said third recombination site (6) and said fourth recombination site (7) do not recombine with each other or with a same recombination site.

2. The vector of claim 1, wherein said first (4) and second recombination site (5) flank a second selectable marker gene (10) and said third (6) and fourth recombination site (7) flank a third selectable marker gene (9).

3. The vector of claim 1, wherein said chimeric DNA construct comprises a region flanked by intron processing signals (11), functional in said eukaryotic cell, located between said second recombination site (5) and said third recombination site (6).

4. The vector of claim 3, wherein said region flanked by intron processing signals is an intron sequence functional in said eukaryotic cell.

5. The vector of claim 3, further comprising a fourth selectable marker gene (19), located between said second (5) and third recombination site (6).

6. The vector of claim 1, wherein said selectable marker genes are selected from the group consisting of an antibiotic resistance gene, a tRNA gene, an auxotrophic marker, a toxic gene, a phenotypic marker, an antisense oligonucleotide; a restriction endonuclease; a restriction endonuclease cleavage site, an enzyme cleavage site, a protein binding site, an a sequence complementary PCR primer.

7. The vector of claim 1, wherein said promoter (3) is a plant- expressible promoter.

8. The vector of claim 7, wherein said chimeric DNA construct is flanked by left and right border T-DNA sequences.

9. The vector of claim 8, further comprising a selectable marker gene capable of being expressed in plant cells located between said left and said right T-DNA border sequences.

10. The vector of claim 8, further comprising an origin of replication capable of functioning in *Agrobacterium* sp.
11. The vector of claim 1, wherein said first (4) and fourth recombination site (7) is attR1 comprising the nucleotide sequence of SEQ ID No 4 and said second (5) and third (6) recombination site is attR2 comprising the nucleotide sequence of SEQ ID No 5.
12. The vector of claim 1, wherein said first (4) and fourth recombination site (7) is attP1 comprising the nucleotide sequence of SEQ ID No 10 and said second (5) and third (6) recombination site is attP2 comprising the nucleotide sequence of SEQ ID No 11.
13. A vector comprising the sequence of SEQ ID No 13.
14. A vector comprising the sequence of SEQ ID No 23.
15. A vector comprising the sequence of SEQ ID No 24.
16. A vector comprising the sequence of SEQ ID No 25.
17. A vector comprising the sequence of SEQ ID No 26.
18. A vector comprising the following operably linked DNA fragments:
  - an origin of replication allowing replication in a recipient cell (1), preferably in bacteria; particularly in *Escherichia coli*;
  - a selectable marker region (2) capable of being expressed in said recipient cell; and
  - a chimeric DNA construct comprising in sequence:
    - a promoter or promoter region (3) capable of being recognized by a prokaryotic RNA polymerase;
    - a first recombination site (4), a second recombination site (5), a third recombination site (6) and a fourth recombination site (7);
    - a 3' transcription terminating and polyadenylation region (8) functional in said eukaryotic cell;wherein
  - said first recombination site (4) and said fourth recombination site (7) are capable of reacting with a same recombination site, preferably are identical; and
  - said second recombination site (5) and said third recombination site (6) are capable of reacting with a same recombination site, preferably are identical;and wherein
  - said first recombination site (4) and said second recombination site (5) do not recombine with each other or with a same recombination site; or
  - said third recombination site (6) and said fourth recombination site (7) do not recombine with each other or with a same recombination site.
19. The vector of claim 18, wherein said RNA polymerase is a bacteriophage single subunit RNA polymerase.
20. A kit comprising the vector of claim 1.
21. The kit of claim 20, further comprising at least one recombination protein capable of recombining a DNA segment comprising at least one of said recombination sites.

22. A method for making a chimeric DNA construct capable of expressing a dsRNA in a eukaryotic cell comprising the steps of combining *in vitro*:

- a vector according to claim 1;
- an insert DNA comprising a DNA segment of interest (12) flanked by
  - a fifth recombination site (13) which is capable of recombining with said first (4) or fourth recombination site (7) on said vector; and
  - a sixth recombination site (14) which is capable of recombining with said second (5) or third recombination site (6) on said vector;
- at least one site specific recombination protein capable of recombining said first (4) or fourth (7) and said fifth recombination site (13) and said second (5) or third (6) and said sixth recombination site (14);

allowing recombination to occur so as to produce a reaction mixture comprising product DNA molecules, said product DNA molecule comprising in sequence:

- said promoter or promoter region (3) capable of being recognized by RNA polymerases of said eukaryotic cell;
- a recombination site (15) which is the recombination product of said first (4) and said fifth recombination site (13);
- said DNA fragment of interest (12);
- a recombination site (16) which is the recombination product of said second (4) and said sixth recombination site (14);
- a recombination site (17) which is the recombination product of said third (5) and said sixth recombination site (14);
- said DNA fragment of interest in opposite orientation (12);
- a recombination site (18) which is the recombination product of said fourth (7) and said fifth recombination site (13); and
- said 3' transcription terminating and polyadenylation region (8) functional in said eukaryotic cell; and

selecting said product DNA molecules.

23. The method according to claim 22, wherein said selecting is carried out *in vivo*.

24. The method according to claim 22, wherein said insert DNA is a linear DNA molecule.

25. The method according to claim 22, wherein said insert DNA is a circular DNA molecule.

26. The method according to claim 22, wherein said at least one recombination protein is selected from (i) Int and IHF and (ii) Int, Xis, and IHF.

27. The method according to claim 22, wherein multiple insert DNAs comprising different DNA fragments of interest are processed simultaneously.

28. A method for preparing a eukaryotic non-human organism wherein the phenotypic expression of a target nucleic acid of interest is reduced or inhibited, said method comprising: preparing a chimeric DNA construct comprising a nucleic acid of interest (12) comprising a nucleotide sequence of at least 19 bp with at least 70% sequence identity to said target nucleic acid capable of expressing a dsRNA in cells of said eukaryotic non-human organism according to the method of claim 22; introducing said chimeric DNA construct in cells of said eukaryotic non-human organism; and isolating said eukaryotic organism

28. The method of claim 28, wherein said eukaryotic organism is a plant.
29. A method for isolating a nucleic acid molecule involved in determining a particular trait comprising the steps of:  
preparing a library of chimeric DNA constructs capable of expressing a dsRNA in cells of said eukaryotic non-human organism according to the method of claim 22;  
introducing individual representatives of said library of chimeric DNA constructs in cells of said eukaryotic non-human organism;  
isolating a eukaryotic organism exhibiting said particular trait; and  
isolating said nucleic acid molecule.
30. The method according to claim 30, wherein said eukaryotic organism is a plant.
31. A eukaryotic non-human organism comprising a chimeric DNA construct obtainable through the method of claim 22.
32. The non-human eukaryotic organism according to claim 31 that is a plant.

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